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JOHNS HOPKINS UNIV BALTIMORE MD DEPT OF ENVIRONMENT--ETC F/6 6/15
BIOCHEMICAL ANALYSIS OF CEREBROSPINAL FLUID CHANGES IN RESPONSE--ETC(U)
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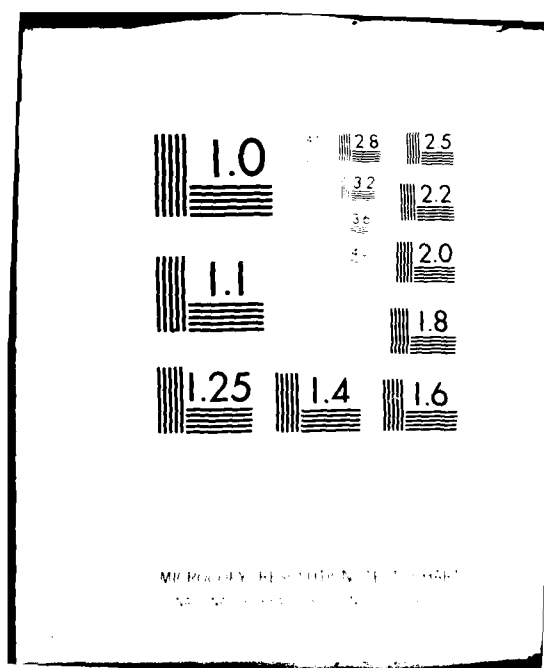
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REPORT NUMBER 02

Final Report

Barry Burns, Ph.D.

11 Sept., 1973

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Washington, D.C. 20314

Contract No. DADA17-72-C-2119

THE JOHNS HOPKINS UNIVERSITY
Baltimore, Maryland 21205

DDC AVAILABILITY STATEMENT

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Joseph V. Brady, Ph. D.

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Summary

The general aim of this program was to develop a monkey model for the evaluation of physiologic and neurochemical effects of some commonly abused drugs. The first two quarters were spent in the preliminary design and testing of chronic devices used for the collection of cerebrospinal fluid (CSF) for analysis of neurotransmitter metabolites. The second two quarters were devoted to the further refinement of the chronic devices and preliminary testing of d- and l-amphetamine and methadone. The drug studies have carried over into the second year of funding and will be presented in great detail in the 1973-74 Annual Report. The findings presented in this report pertain to the amphetamine experiments in M. mulatta and a brief overview of the effects of methadone on the circulation in acute studies on dogs.

For the primate studies, we have successfully designed a chronic, remote sampling system with minimal dead space (<0.06 cc). Using this system we were able to sample either cisternal or ventricular CSF at regular intervals or continuously before and after drug administration. The experimental apparatus consisted of an isolation booth provided with white noise, light and ventilation. Behavior was monitored and recorded with an Ampex video-tape, and the CSF is now collected automatically in a refrigerated fraction collector at 4 - 10°C, mounted to the back of the booth.

Additional physiologic measurements of respiration, blood pressure and blood gas data (pH, PO₂, PCO₂) have been incorporated into the chronic monkey preparation by the use of indwelling catheters in the internal jugular vein and femoral artery, and a strain gauge on the thorax to measure respiratory frequency and tidal volume. At the outset these measurements appear to have been extremely useful in evaluating the nature of drug action in the intact animals. The circulatory studies on dogs were begun during the last quarter in order that more extensive measurements of central and peripheral circulatory factors could be undertaken. Both methadone and metyrapone seem to exert a selective direct action on the myocardium, independent of any CNS effects. These data are discussed in the Supplement to the body of the report (see Appendix A).

Only amphetamines were tested with the primates, and the first series involved the administration of both the d- and l-stereoisomers which are asymmetric at the α -carbon. Both isomers at a dosage of 1.5 mg/kg exhibit marked peripheral respiratory and cardiovascular effects. Only the d-isomer however results in behavioral stereotypy (licking, gnawing and backward looking movements). We found in these experiments that the levels of the dopamine metabolite HVA tended to decrease after the d-isomer, and either increased or stayed the same after the l-isomer at the same dosage level. The serotonin metabolite 5-HIAA increased and then decreased slightly after both isomers, but these changes were not significant.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences, National Research Council.

The following personnel were supported by this contract:

Barry Burns	Principal investigator	SS# 462-72-3434
Joseph V. Brady	Co-investigator	057-14-6665
Dick Rabold	Technician	174-42-7011
Kris Martin	"	136-38-4585
Gilbert Eidman	"	220-52-2671

PUBLICATIONS: - none

TABLE OF CONTENTS

SUMMARY.....	1
LISTS OF APPENDICES, ILLUSTRATIONS, TABLES.....	4
PROBLEM.....	5
BACKGROUND.....	5
EXPERIMENTAL APPROACH.....	6
RESULTS.....	8
DISCUSSION.....	27
CONCLUSIONS AND RECOMMENDATIONS.....	33
LITERATURE CITED.....	34
ABSTRACT (DD Form 1473).....	39

LISTS OF APPENDICES, ILLUSTRATIONS AND TABLES

	<u>Page</u>
Figure 1 CSF sampling techniques.....	9
Figure 2 Chronic head-mounted column.....	10
Figure 3 Remote sampling device for CSF.....	11
Figure 4 Experimental chamber.....	12
Figure 5 Implantable devices; effects of catheter coating.....	14
Figure 6 Arterial pressure measuring system.....	16
Figure 7 d-Amphetamine effects on behavior, circulation, heart rate, respiration and CSF biochemical changes in HVA, 5-HIAA.....	18
Figure 8 l-Amphetamine effects on behavior, circulation, heart rate, respiration and CSF biochemical changes in HVA, 5-HIAA.....	19
Figure 9 Control levels of HVA in CSF versus response to a single dose of l-amphetamine.....	20
Figure 10 Control levels of HVA in CSF versus response to a single dose of d-amphetamine.....	21
Figure 11 Control levels of 5-HIAA in CSF versus response to a single dose of d-amphetamine.....	22
Figure 12 Control levels of 5-HIAA in CSF versus response to a single dose of l-amphetamine.....	23
Figure 13 Circulatory and respiratory recording of response to a single dose of l-amphetamine.....	24
Figure 14 Circulatory and respiratory recording of response to a single dose of l-amphetamine (expanded time scale).....	25
Figure 15 Circulatory and respiratory recording of response to a single dose of d-amphetamine.....	26
Figure 16 CSF levels of HVA and 5-HIAA: Serial d-amphetamine injections.....	28
Figure 17 CSF levels of HVA and 5-HIAA after Probenecid.....	29
Figure 18 CSF levels of HVA and 5-HIAA after Probenecid twice....	30
APPENDIX - A	35
Figure 19A,B Effects of methadone; metyrapone on circulation....	36

BODY OF REPORT

PROBLEM

- A. Design and test various types of sampling devices for obtaining CSF in a chronic monkey preparation. Refine existing techniques.
- B. Evaluate materials and coatings for implantation within the ventricles and sub-arachnoid space of the brain.
- C. Establish procedures for handling and storage of CSF prior to analysis.
- D. Measure the CSF neurochemical response to drug administration and correlate these changes with behavior and cardiovascular effects.

The general aim of this contract work was to develop a useful monkey model which could be used to ascertain the physiologic, neural biochemical and behavioral correlates of drug action. The ultimate goal is to generate information which may have some practical application in the field of drug abuse.

BACKGROUND

There are a number of methodological difficulties which arise during repeated sampling of CSF in chronic animals. Foremost among these is the significant dead space in most systems, compared with the volume of CSF which can be withdrawn discretely. It has also been observed that indwelling ventricular or sub-arachnoid catheters will fibrose in less than two or three weeks time (Ommaya, personal communication). Another method involving the insertion of a device through the brain preceding the CSF sample has routinely led to spreading ependymitis and gradual occlusion of the ventricle by scar tissue (Pappenheimer, et al., 1962; Myers, et al., 1967; Ashcroft et al., 1968). The importance of these methodological problems cannot be overemphasized since their satisfactory resolution is prerequisite to studies of CSF during drug administration.

Clearly, drug addiction and abuse are a function of several variables related to the biochemical nature of certain compounds and the nature of the total drug effect on the physiology of the species being studied. The basic biological effects of a drug are key to understanding preference, abuse and addiction. In the initial contract proposal we planned to study the sequence of neural-biochemical events associated with drug administration in unanesthetized primates, but emphasized that existing techniques for sampling CSF were inadequate and were inconsistent with methods for measuring behavioral and cardiovascular effects simultaneously.

We felt that if reliable samples of CSF could be obtained remotely from chronically prepared monkeys maintained in an isolated environment so that interactions with the experimenter would not interfere with the normal drug effects, we would be in a position to determine drug interaction with a number of different neurotransmitter systems simultaneously and conduct repeated experiments on the same animal to better compare the different effects. Although not specifically implicated in the original proposal, we have incorporated cardiovascular parameters in these drug studies because of their value in relationship to the central norepinephrine transmitter systems controlling blood pressure, heart rate and respiration. These measures have proved valuable in evaluating drug action, and we would like to expand on these and continue this type of measurements in future research efforts.

EXPERIMENTAL APPROACH

Problem A. To test the various sampling devices shown in Fig. 1, we had head mounted columns of our own designs machined from a variety of different materials (stainless steel, lucite and Nylon 101). The stainless steel devices were chosen for their ruggedness, and the final designs utilized nylon to compensate for the adverse tissue reaction to the stainless steel. We also tried to design a column which would prevent tissue fluids from seeping up the threads on the cap, resulting in infection in the CSF and difficult removal of caps to change septums periodically.

Additional concerns involved the type of rubber used in the septums which permitted repeated sterile access to the CSF in Fig. 1A,B,D. Usually the materials were only able to withstand a limited number of penetrations before they began leaking.

Although the subcutaneous reservoirs were not the method of choice for these studies, we did attempt to refine on this technique also by reducing the size of the reservoir and varying the actual placement (Fig. 1C,E).

The problem of residual dead space was approached from two directions: first, we attempted to minimize the actual dead space in the column by reducing the dimensions in the new designs; secondly, we switched to the use of small I.D. silicone rubber tubing to connect the columns to the CSF compartment and to exit from the booth from the top of the column.

A major part of the refinement of existing experimental techniques involved placing the monkey in a booth and making all measurements remotely to reduce the contribution of extraneous variables to the observed responses.

Problem B. In an attempt to increase the tissue compatibility of the chronically implanted devices within the ventricles or sub-arachnoid space, we attempted to reduce a) the mechanical irritation by the use of flexible silicone rubber catheters, and b) the rate of tissue overgrowth on the implanted catheters by the use of a surface coating which imparts a strong electronegativity. It is well known that the precipitating factor for thrombus formation at vessel walls is the presence of a positive charge resulting from tissue injury. Most mammalian cells maintain a net negative charge on the outer cell wall, and we reasoned that the process of tissue adherence to a catheter could be accentuated by positively charged devices

and minimized by negatively charged devices. To impart a surface negativity, we used TDMAC and heparin - a process developed by the Battelle Columbus Laboratories for intravascularly positioned catheters. This coating has not been tested on chronic devices in the brain to date, and so far as we know, ours is the only work of this type. TDMAC or the TDMAC/heparin complex can be purchased from Polysciences, Inc., Paul Valley Industrial Park, Warrington, Penna., 18976.

As an effort to sidestep the catheter occlusion problem we have tested semi-chronically implanted stainless steel tubes in the ventricles (Fig. 1A). These tubes are only removed and cleaned when they become clogged and are not inserted prior to each sample withdrawal as has been customary with other investigators.

Problem C. We have investigated the possibility of storing samples in liquid nitrogen by purchasing and evaluating a 30 liter storage container from Union Carbide. This container is capable of holding several hundred small vials on individual canes, and requires minimal space in the laboratory. The extremely low temperature minimizes any loss of enzymatic activity or degradation of samples.

We have streamlined the handling procedure by the use of disposable test tubes and pipette tips whenever possible, reducing the time spent washing glassware, etc. The actual storage of samples in small screw-top vials and heat sealable glass ampoules has been worked out. These vials and ampoules are acid-washed prior to use to minimize possible contamination.

Problem D. In order to measure the behavioral changes of drug administration we have incorporated the use of a recording AMPEX VR500 videotape for permanent behavior records. The videotape records are replayed and serial observations are made on the behavioral changes by two people independently following the experiments. We rate such things as general restlessness, licking and gnawing movements and any other stereotyped behavior (backward looking motions, etc.).

To assess the CSF neurochemical response, we collect 0.5 - 1.0cc aliquots of CSF remotely from the animal two hours before and 5 hours after the administration of the test drug. Control days are run prior to and between drug days, and CSF samples are taken at hourly intervals and assayed for the same neurotransmitter metabolites.

We have incorporated the measurement of blood pressure, heart rate and respiratory frequency and tidal volume into the measurement of the drug response. Although these parameters were not specified in the original contract, it is apparent that they are extremely valuable in determining the nature of the drug response, and they give additional information of activity in neural circuits whose transmitter metabolites we do not assay the CSF for. In order to measure respiration, a sensitive strain gauge is permanently strapped to the thorax. Blood pressure and heart rate are obtained by means of a chronic femoral artery catheter (Fig. 5), and the drugs are administered by a similar catheter in the internal jugular vein (not shown in Fig. 5). Prior to and during the experimental run, the monkey is maintained in a sound-proofed, lighted, ventilated booth (Fig. 4) which has a small access window through which the videotape camera is directed.

RESULTS

Problem A. We have refined the design of the head mounted columns used to sample ventricular or cisternal CSF. The final design is shown in Fig. 2. The columns machined from stainless steel seemed to undergo a continual sort of electrolysis in contact with the body fluids, and resulted in a significant tissue reaction and oozing at the contact points. This may be due in part to the presence of a potential difference of 5-15 mv between the CSF and the tissues outside the brain. This potential difference would act to erode the steel and change the local ionic environment, resulting in the tissue reaction. Whatever the reason, the use of Nylon 101 eliminates these problems. This material is autoclavable and easily machinable. Columns of lucite did not have sufficient strength and tended to break off easily, ruining the preparation and usually creating CNS infections because the damage was not detected for periods of several hours or until the following day.

To sample CSF directly from the column in Fig. 1B or 1D, the septum is merely penetrated with a sterile needle through the hole in the top of the cap after the area is cleaned with 95% ethanol, and the CSF drawn into the attached syringe. It is necessary to use needles smaller than 27 1/2 ga, otherwise relatively few penetrations destroy the septum integrity and may lead to infection. Using a 30 ga. needle, the septums are good for several weeks of repeated penetrations.

To sample the CSF remotely from the columns in Fig. 1A, 1B and 1D, a length of PE20 or PE10 tubing is connected to the end of a short needle which protrudes from the top of the column distal to the monkey and penetrates the septum at the other end. The needle and tubing system may be positioned in the morning and left all day if desired, allowing repeated or continual access to CSF without disturbing the monkey. In Fig. 1A, the tubing is attached to the distal end of the ventricular guide by means of a short length of silicone rubber adapter 7mm in length. A more detailed representation of the ventricular sampling system is shown in Fig. 3. The alternate side hole exit design in Fig. 2 is used for the sampling methods represented by Fig. 1B and 1D, in which indwelling catheters are permanently placed in either the cisterna magna or in the lateral ventricle.

Using the subcutaneous reservoir depicted in Fig. 1C and 1E vastly simplifies the maintenance of the animals, and permits them to be housed in a cage instead of a primate chair, but the dead space is excessive (greater than 1.5 cc) and precludes their use when small samples of CSF are being taken.

Whenever the CSF is being sampled remotely, or we are running a drug study, the animal is placed in the booth depicted in Fig. 4. The CSF is collected through a hole directly in back of the monkeys head, with appropriate precautions to ensure that the tubing exits are air-tight and sound proofed. The CSF catheter is terminated in a needle hub and luer adapter and the samples are withdrawn into sterile lcc syringes over periods of 2-3 minutes to minimize the rate of volume change. If too much is removed, the animals become obviously distressed, as observed on the videotape monitor and by variations in blood pressure and respiration.

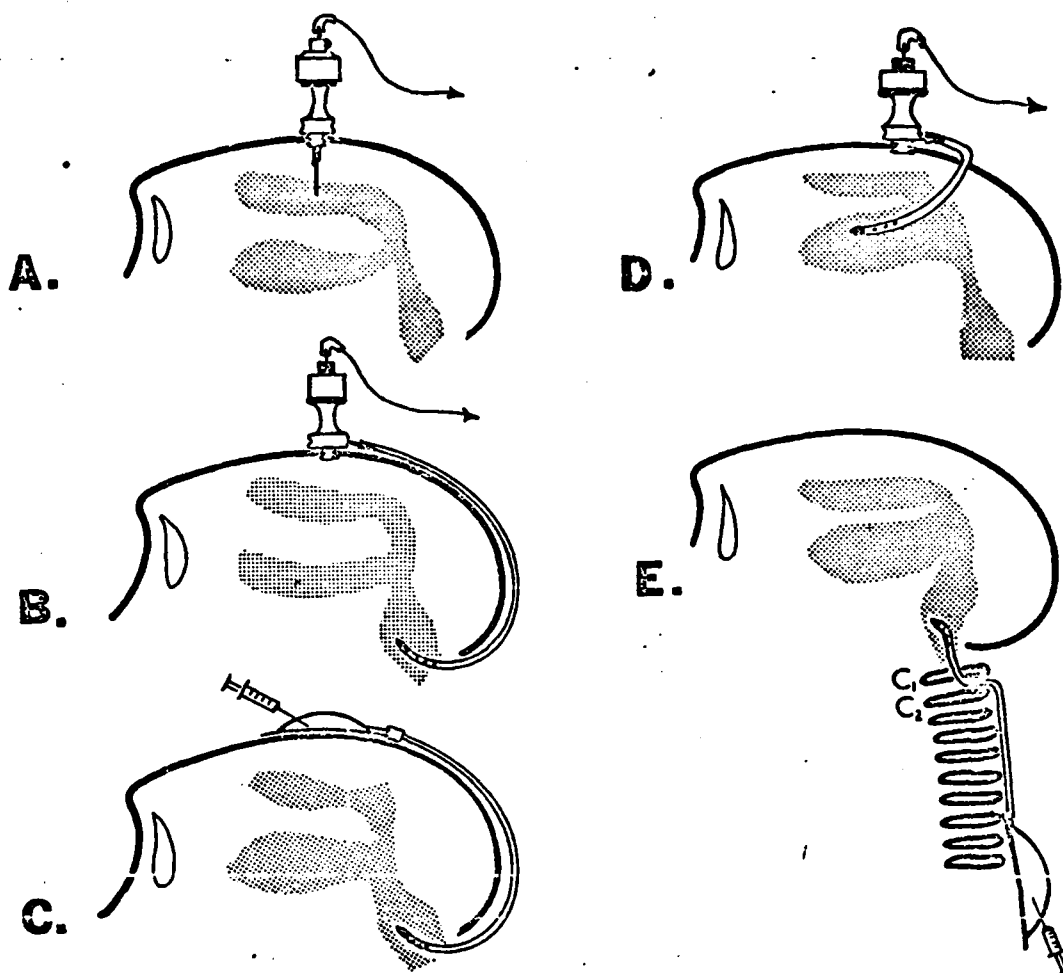


FIGURE 1. This figure depicts the various types of sampling techniques for obtaining CSF evaluated during this contract period. An approximate outline of the ventricular system from lateral to cisterna magna is represented by the shaded area. The column in (A) was designed for continuous or repeated access to the lateral ventricle. Whenever the sampling tube becomes clogged with debris or cell growth it is removed, cleaned, autoclaved and replaced. It will normally function for 1- 2 weeks between removals and for up to 4 months for continual use with intermittent removal and cleaning. Depth of penetration is controlled by an adjustable stop on the sampling tube and the septums are changed every 1- 2 weeks. (B) represents an alternate design with a side-hole exit to which is attached the tubing leading to either the cisterna magna or the lateral ventricle (D). The subcutaneous Ommaya reservoir is depicted in (C), connected to a cisternal catheter and positioned on the top of the head. Somewhat easier access is guaranteed if the reservoir is located on the lower back region as in (E), not requiring immobilization of the head to collect samples. Dead space is appreciable and the reservoirs must be manually flushed several times a day to ensure freedom from infection in the large dependent dead space and to enable the contents to accurately reflect changes in the composition of the CSF. The flushing also tends to blow small bits of debris or adherent cells from the side holes at the catheter tip in the CSF, but must be done slowly.

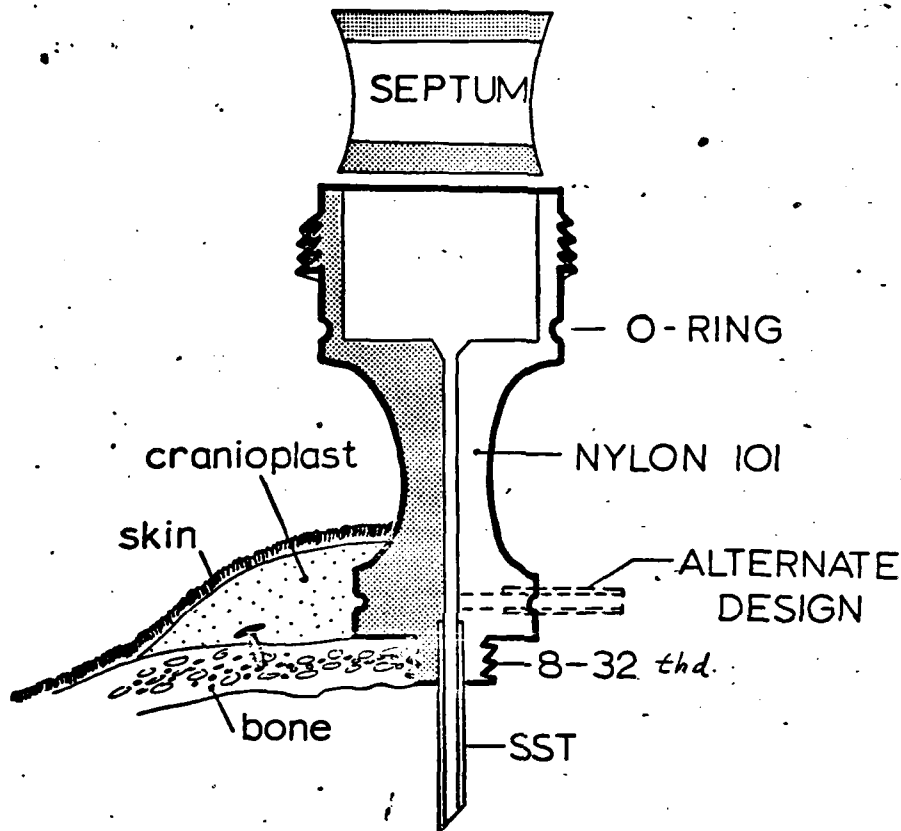


Fig. 2 Chronic head mounted column(cap not shown). Used for either ventricular or sub-arachnoid(alternate design- see Fig. 1B) CSF collection.

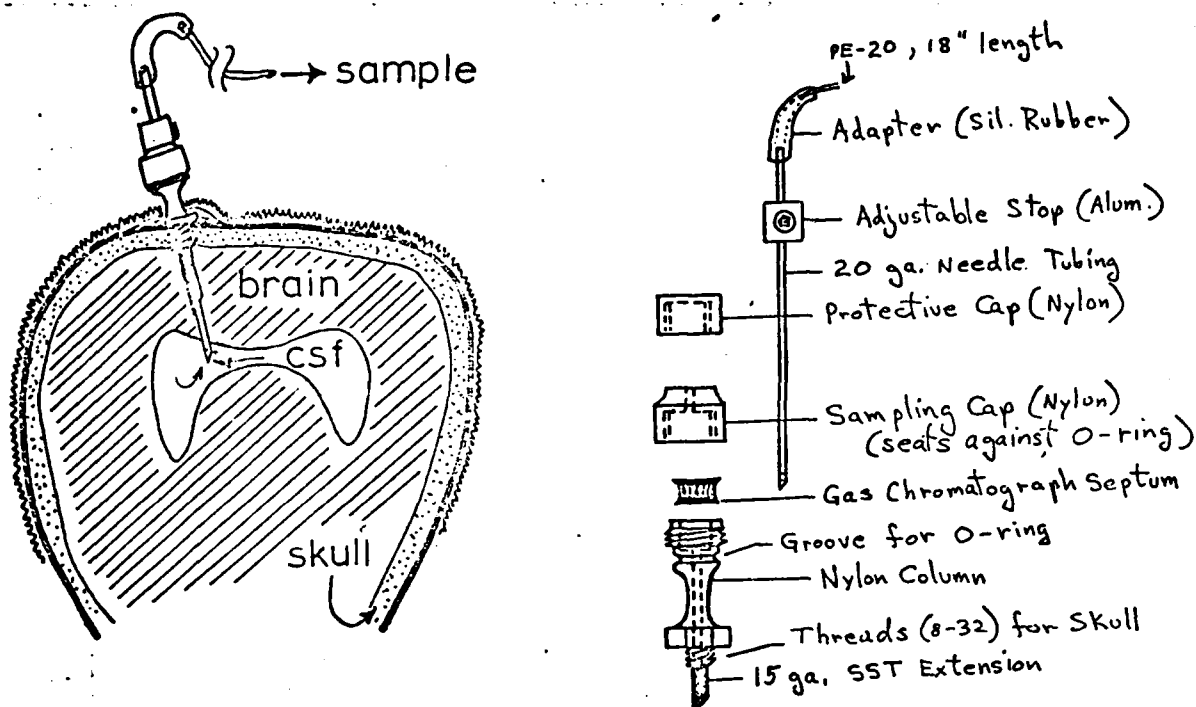


FIGURE 3. Frontal section through the monkey head, showing the nylon column and sampling tube in position for remote access to the ventricular CSF. The sampling tube has small side holes at the tip to assist in the removal of CSF; however in a period of 1 - 2 weeks, these will become clogged by tissue growth and must be removed, cleaned, autoclaved and re-inserted. Depth of penetration may be varied by adjusting the stop using the allen screw in the side. During insertion of the sampling tube, an inner obturator is positioned to prevent the entry of rubber septum material or tissue during the actual insertion. The obturator is removed and the tubing harness (sterile) attached and led from the experimental chamber. CSF is then withdrawn or solutions infused through this device. The column base is attached to the skull by means of a threaded hole and extended base of cranioplast. The cranioplast is additionally anchored by 3-4 small SST screws anchored to the skull. A sterile tap is used to thread the skull also, prior to inserting the column; this helps to anchor and center the column as the cranioplast is applied.

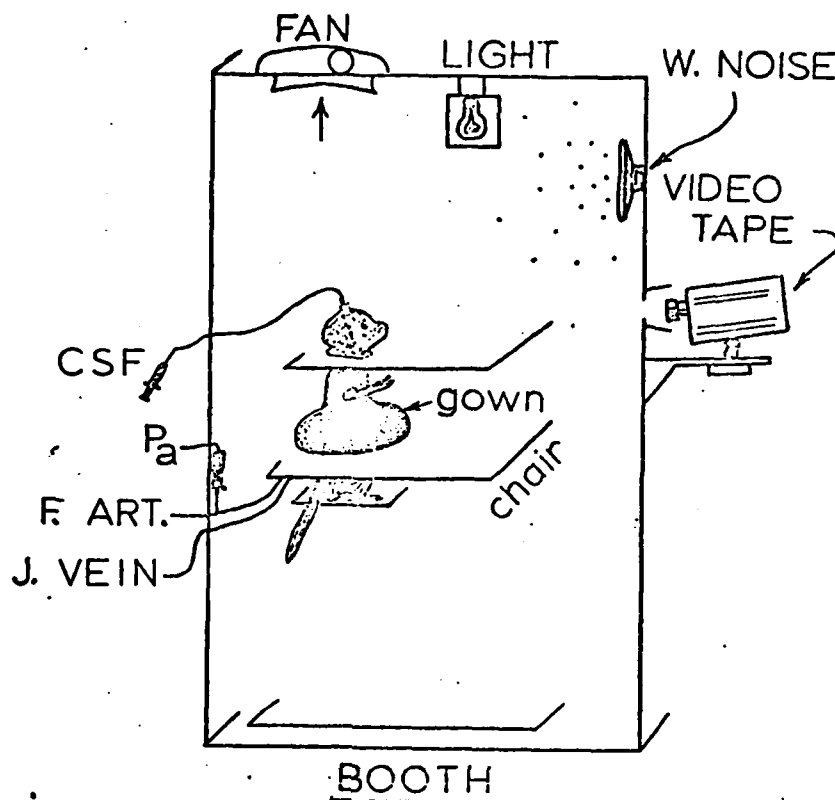


FIGURE 4. Experimental booth which houses the chair and primate during drug studies. The light is attached to a timer, and the monkey may remain in the booth for 24 hr at a time. A small transparent window is provided for the video tape camera. The arterial pressure gauge (P_a) is mounted on the booth in a fixed position with respect to the monkey. The chair is set on rails which permit removal from the booth horizontally for minor adjustments, etc.

RESULTS

Problem B. We have evaluated several different designs of silicone rubber catheters for placement in the ventricles or cisterna magna. These catheters are shown in Fig. 5B. In general, the smaller the diameter of the catheter the less likely is the possibility that it will fibrose and gradually occlude. The open-ended catheters are best for long term use because tissue in the lumen is easily discharged with a vigorous flush. The side hole catheters on the other hand will fail permanently if tissue or debris lodges in the lumen, since the holes are too small to admit particles approaching the lumen diameter. The side hole catheter has some advantages in that CSF flows more readily because the meninges cannot effectively act as valves at the catheter tip - the holes are too numerous.

Catheters with radiopaque markings near the tip are to be preferred because location is possible through simple X-ray. Often, catheter tips may change position due to normal head movement and binding of the catheter in the neck muscles or subcutaneous fascia, and it is important to be able to readily locate the tip if there is any question.

We choose silicone rubber for another reason also: it is easily impregnated with the TDMAC compound which imparts the strong surface negativity. In addition, it is flexible and normally well tolerated by tissues.

The use of the TDMAC-Heparin complex on catheters implanted in the sub-arachnoid space significantly reduces the adherent tissue growth (Fig. 5C), whether the subcutaneous reservoir or the head mounted column system is being used. This finding may be the most significant technological advance of the contract effort, and it certainly enhances the experimental methodology for routine collection of CSF. By increasing the catheter life, it may be possible to study many more drugs using the same monkey, and at the same time allow sufficient time between the individual exposures for complete physiological recovery. The advantage is that each animal can serve as its own control and the total statistical variations reduced by using the same animal to test several drugs successively.

For ventricular placement, we have settled on a specially made ultra small I.D. silicone rubber catheter with open or blunt (side-holes) tip (Fig. 5B, 4th from left). This catheter minimizes the dead space and may be connected to either the head-mounted column or the 1.5cc subcutaneous reservoir in Fig. 1D and 1C, respectively.

Problem C. Sample storage has been achieved in liquid nitrogen. This is especially convenient because the storage container holds a 30 l charge for 90 days in the laboratory. We are not therefore concerned with electrical power failures, and the container is easily moved for routine laboratory cleaning. The extreme low temperature should be effective in preserving activity in the samples of CSF, however this has not been systematically evaluated to date. We may look at this in the future. Vial and ampoule breakage is non-existent with this system to date. As indicated previously, disposable tubes, pipettes, etc., are used whenever possible to streamline the CSF collection procedures.

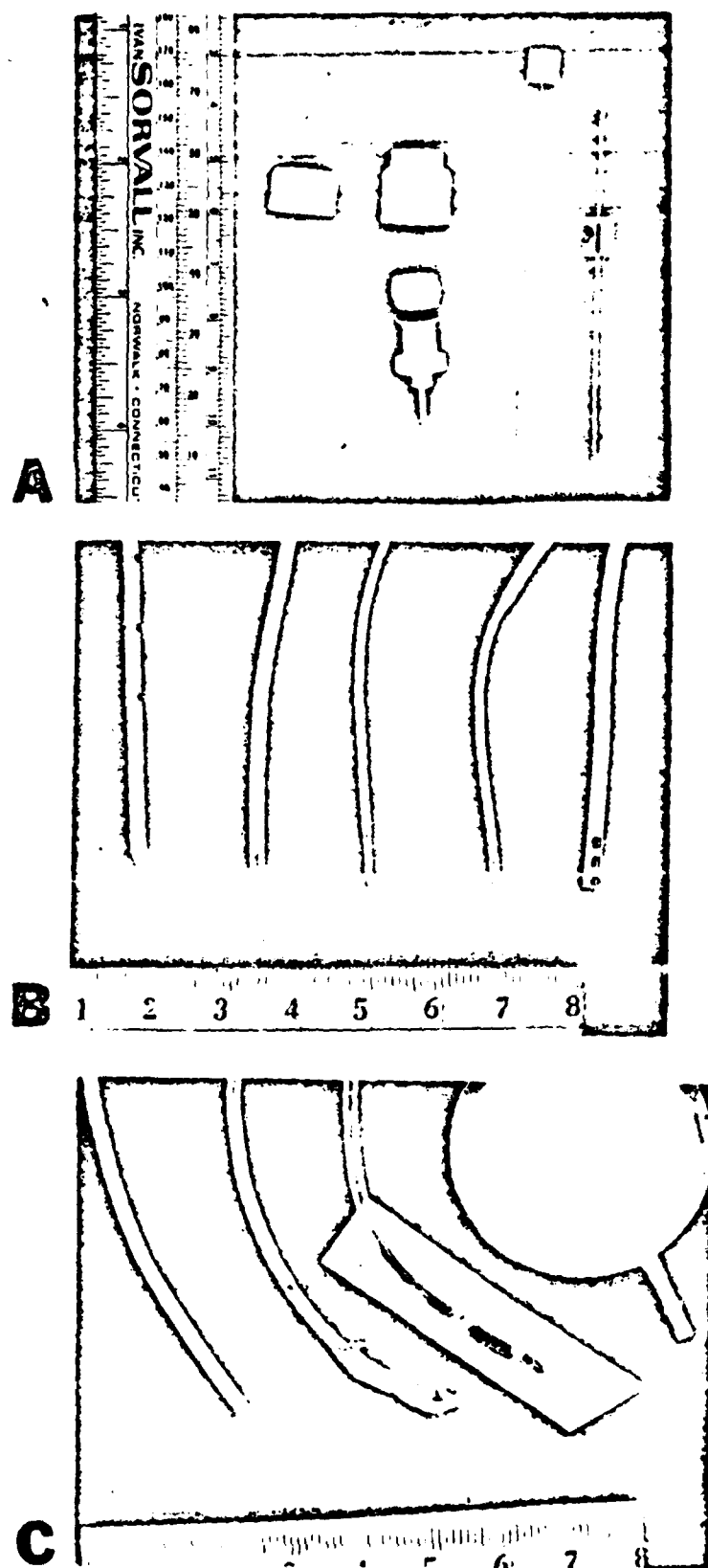


FIGURE 5. Types of chronic devices used for the collection of CSF in the chronic monkeys. (A) depicts the ventricular column and sampling tube

(FIGURE 5, contd.)

shown in Fig. 1A. Both the protective cap and the sampling cap are indicated, as is the obturator used for insertion of the sampling tube with its adjustable stop. The entire unit is steam autoclavable. Septum is not shown. (B) The various catheter types evaluated are from left: standard Mathews ventricular catheter, side holes, radiopaque tip; Pudenz ventricular catheter, side holes, radiopaque tip; small diameter cardiac catheter; special order ultra-small Pudenz-type catheter, side holes, radiopaque tip; open-ended silicone rubber tube with 3 radiopaque dots at tip for localization by X-ray. The last two catheters on the right are preferred for chronic implantation, after coating with TDMAC and heparin to reduce tissue adherence. (C) Evaluation of long-term cisternal implantation of the standard Pudenz silicone rubber catheter with and without the surface negativity. Left: new, unused catheter. Center: untreated catheter after 8 weeks. Right: catheter treated with TDMAC/heparin complex after 8 weeks. Note the complete absence of adherent tissue. Both implanted catheters were carefully dissected from the cisterna magna post-mortem, and they appeared in the animals as they are shown here. Both catheters were connected to the subcutaneous Ommaya reservoir for the duration of implantation in two separate animals. We note that the treated catheters must be gas autoclaved at room temperature to prevent decomposition of the surface-negative coating.

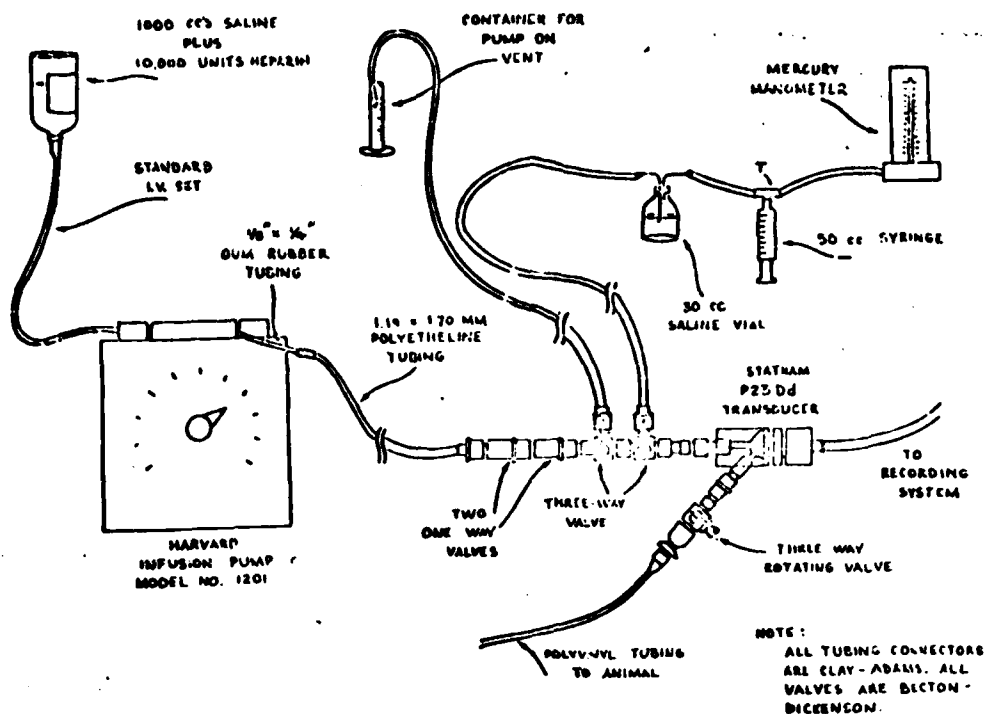


FIGURE 6. System for measuring arterial pressure and providing a constant infusion of saline through both arterial and jugular venous (not shown, but similar) catheters. The pressure transducer is mounted on the booth as shown in Fig. 4. We have tried various concentrations of Heparin in the infusion fluid, and at an infusion rate of 200 cc/day, a minimum Heparin concentration seems to be 1000 Units/liter saline. In some studies, we have infused lactated Ringers solution instead to maintain the plasma ionic balance. Drawing courtesy of D. Randall.

RESULTS

Problem D. Although not specified in the original contract proposal, we decided to include the measurement of arterial blood pressure, heart rate and respiration/tidal volume as dependent variables in the drug response. We did this for two reasons: First, the presence of a chronic venous catheter greatly facilitated the administration of drugs without disturbing the animal; secondly, we wanted to use blood pressure and heart rate as indicators of the general condition of the animal during the amphetamine toxicity studies. We hoped that by monitoring these variables, we would be able to judge better the ability of the animal to tolerate additional amounts of drugs, and avoid accidentally killing the monkey and ruining months of work and preparation.

The interest of the principal author in circulatory and respiratory physiology provide the basis for including these measurements in the future proposed contract work. We believe that our monkey preparation is unique in the wide range of parameters which we are able to study at one time, enhancing the scientific value of the proposed work and at the same time opening new avenues for research on drug abuse problems. The arterial infusion system and pressure measuring device are depicted in Fig. 6. The internal jugular venous catheter is similar to the arterial catheter, with the exception that pressure is not measured. A further description of the circulatory catheters is provided in the renewal application.

Although we do not have a recorder in the laboratory, we did borrow one for a few days for the express purpose of making the tracings shown in Figs. 13-15. We are requesting specific funding to purchase a multi-channel recorder in the renewal request. Practically all of the proposed work, including measurement of CSF production rates will depend on the acquisition of this item. We have been fortunate in acquiring from other sources an Ampex VR500 videotape unit, and an Ampex 7-channel FM tape recorder which will permit replay of some selected data into the departmental PDP-12 computer for subsequent analysis of such things as myocardial contractility, total peripheral resistance, tidal volume, stroke volume (LV) and brain blood flow averaged over 5 sec. periods.

We found that both the d- and l-amphetamine isomers affected the cardiovascular; respiratory parameters more or less equally at first glance (Figs. 7,8). This may indicate a lack of stereoselectivity for the norepinephrine neurons mediating these peripheral responses.

Insofar as the behavioral changes are concerned, only the d-isomer induced stereotyped behavior at dosage levels of 10 mg/I.V. A general restlessness was evident with both isomers, and was accompanied by increased respiratory rate, decreased tidal volume (Fig. 14) and more or less apparent agitation. The stereotyped behavior induced by the d-isomer consisted of a sequence of licking, gnawing and backward-looking behavior repeated every 3-5 seconds and beginning 25 min after the drug administration. No such behavior was ever apparent following the l-isomer at similar dosage levels.

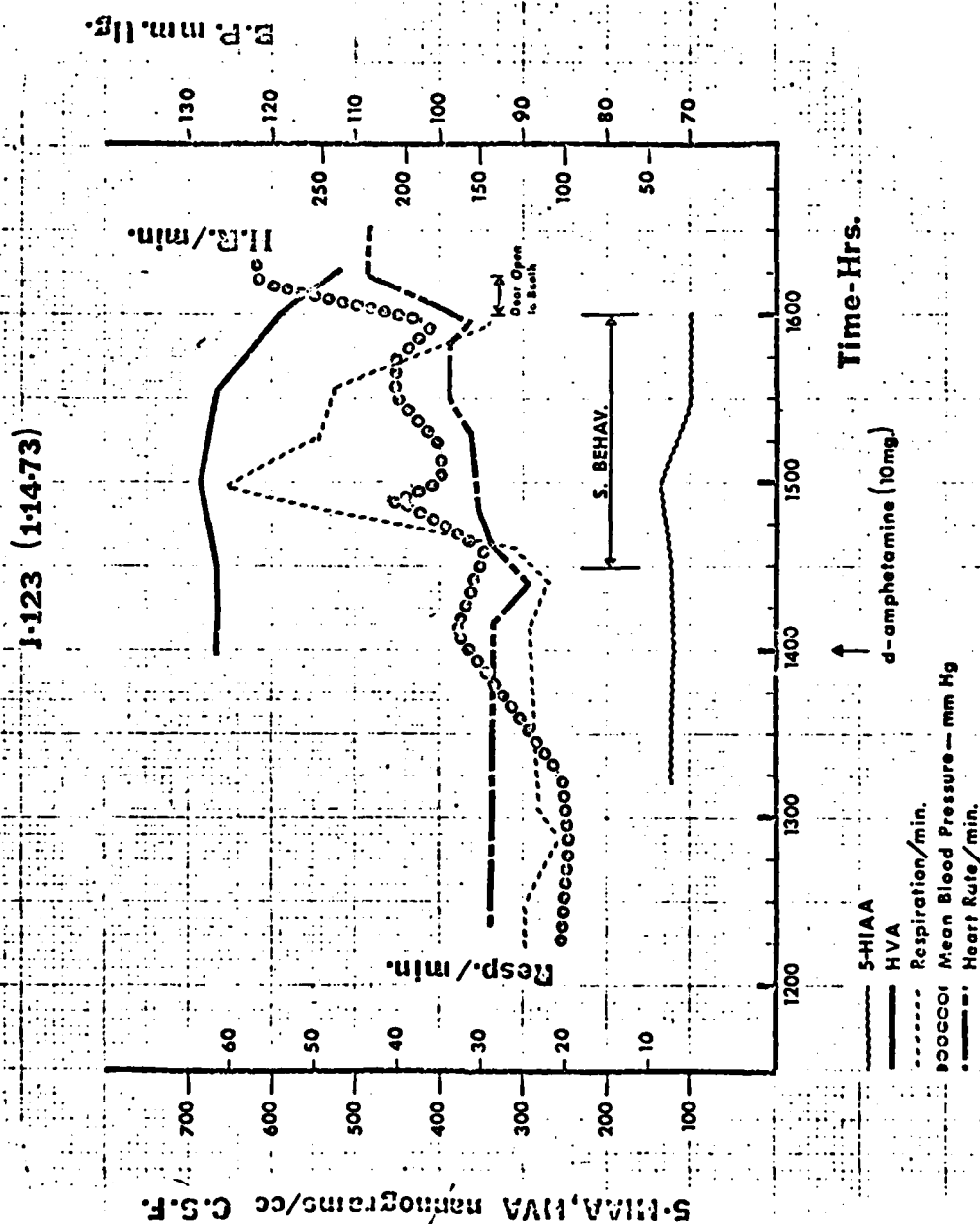


FIGURE 7. Effects of a single dose of d-amphetamine (1.5 mg/kg) on CSF, behavior and circulation/resp.

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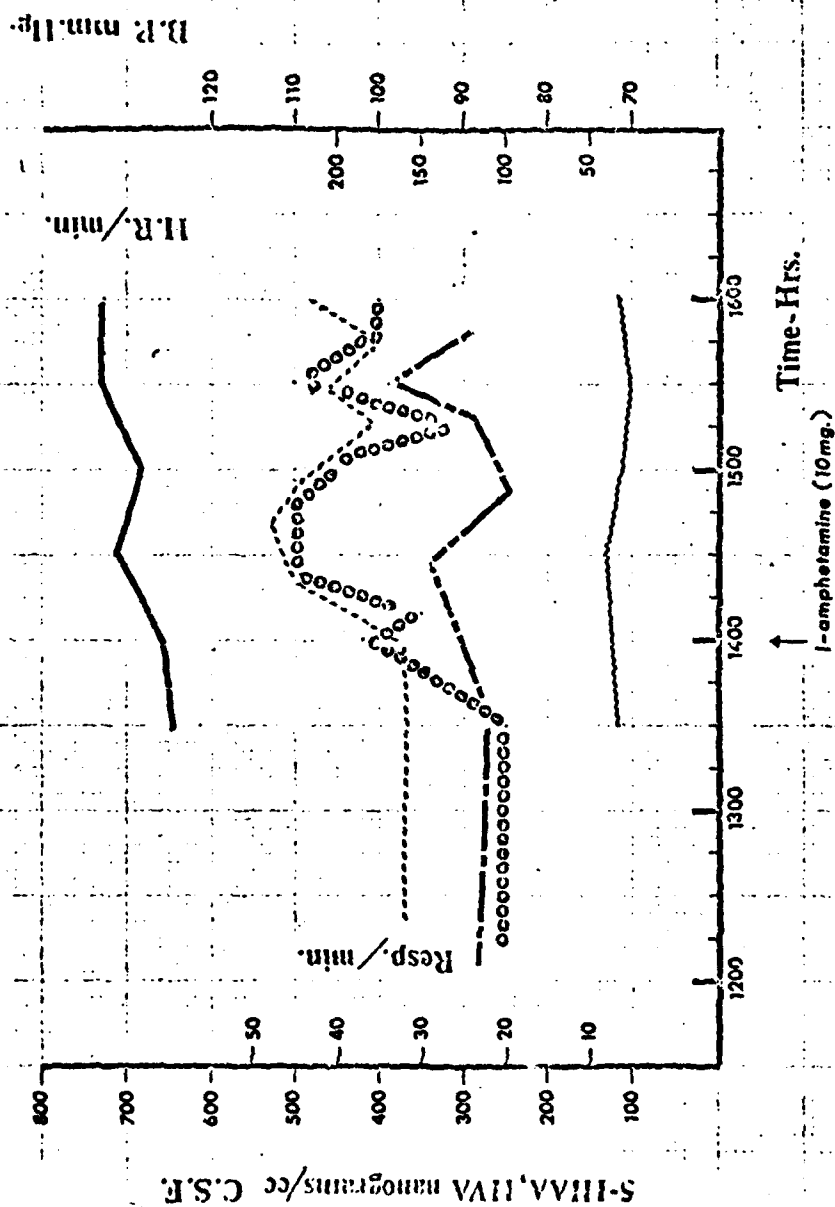


FIGURE 8. Effects of a single dose (1.5 mg/kg) of 1-amphetamine on CSF and cardiovascular system.

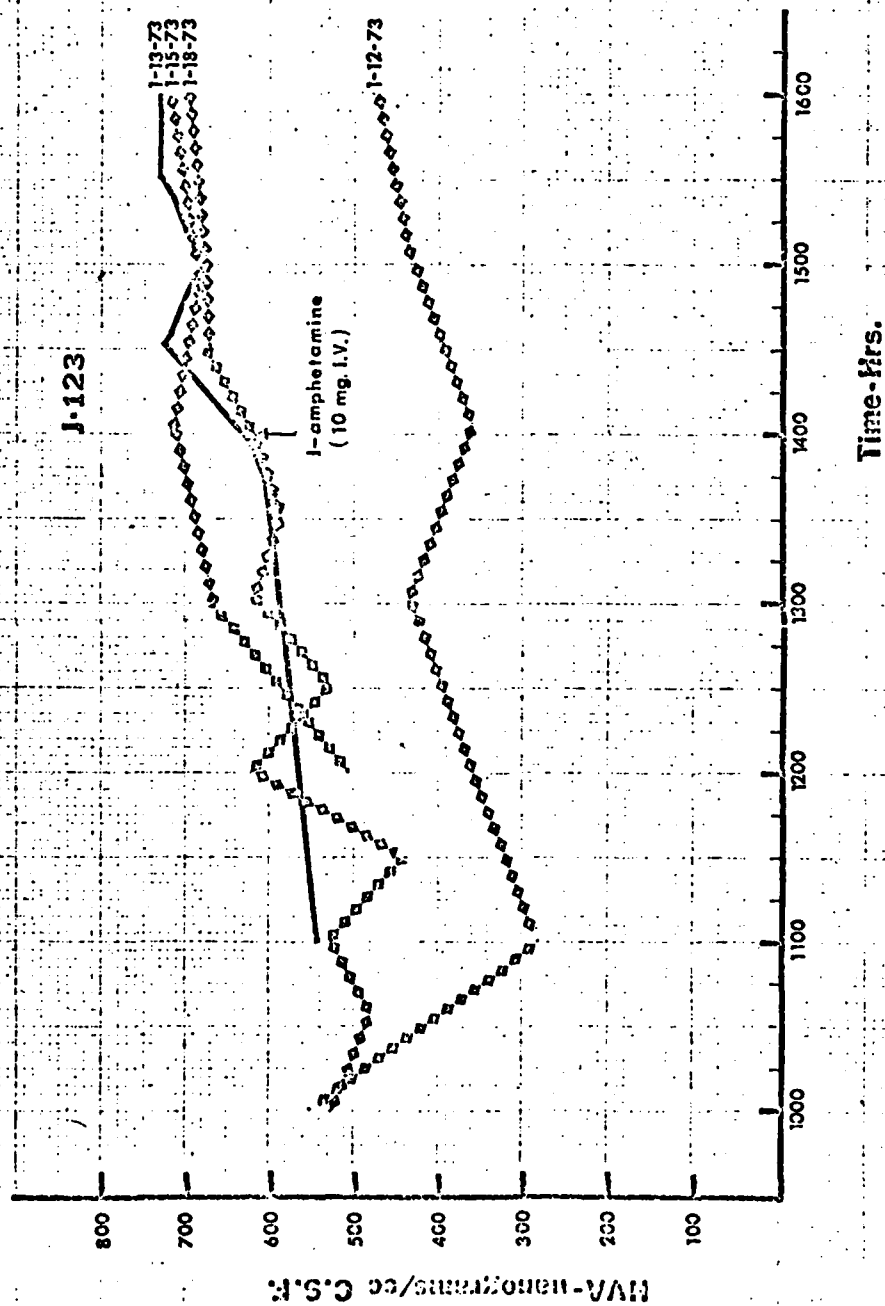


FIGURE 9. A comparison of control days and drug days in the HVA response to 1-amphetamine.

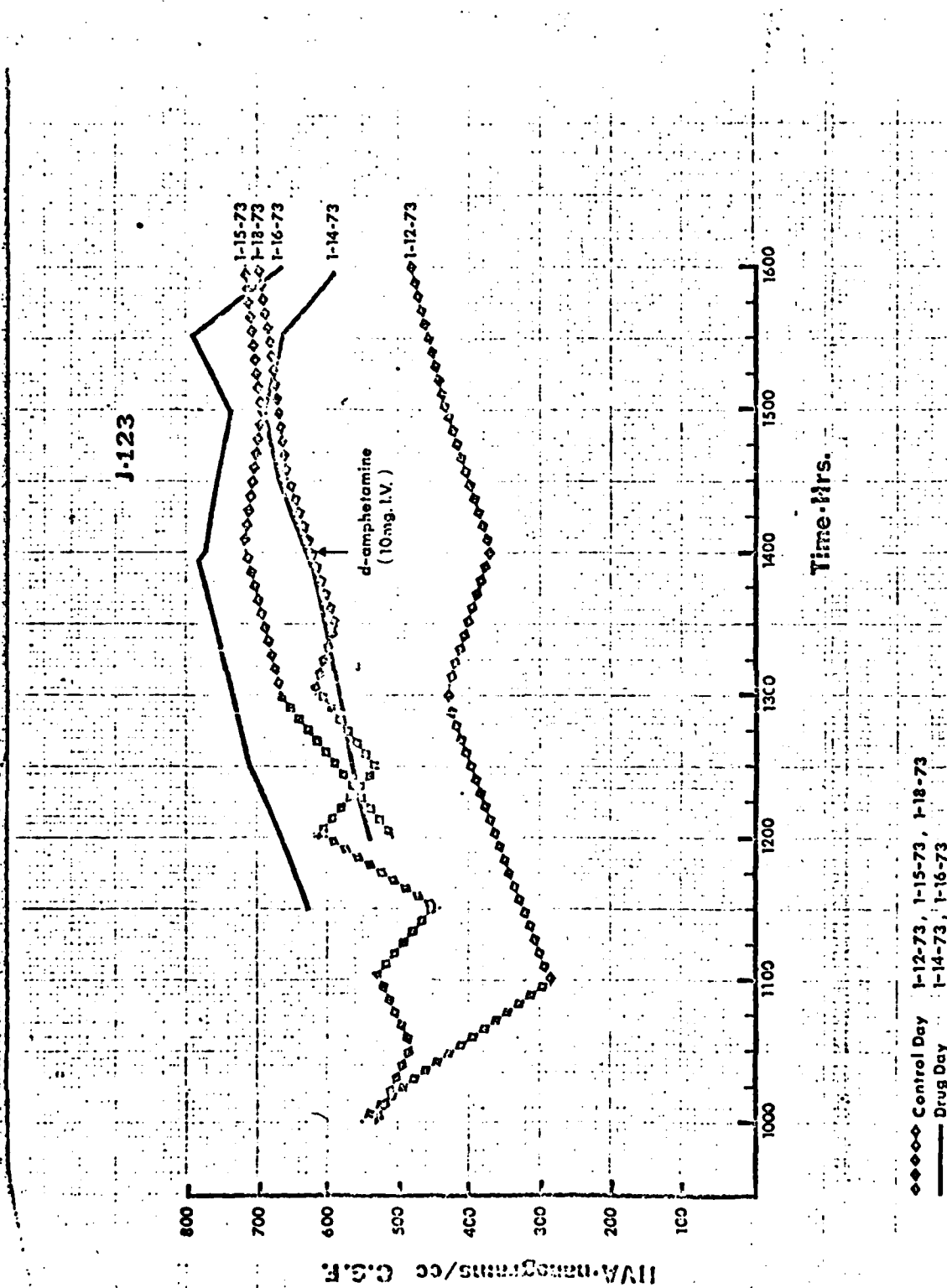


FIGURE 10. A comparison of control days and drug days in the response to d-amphetamine in two experiments.

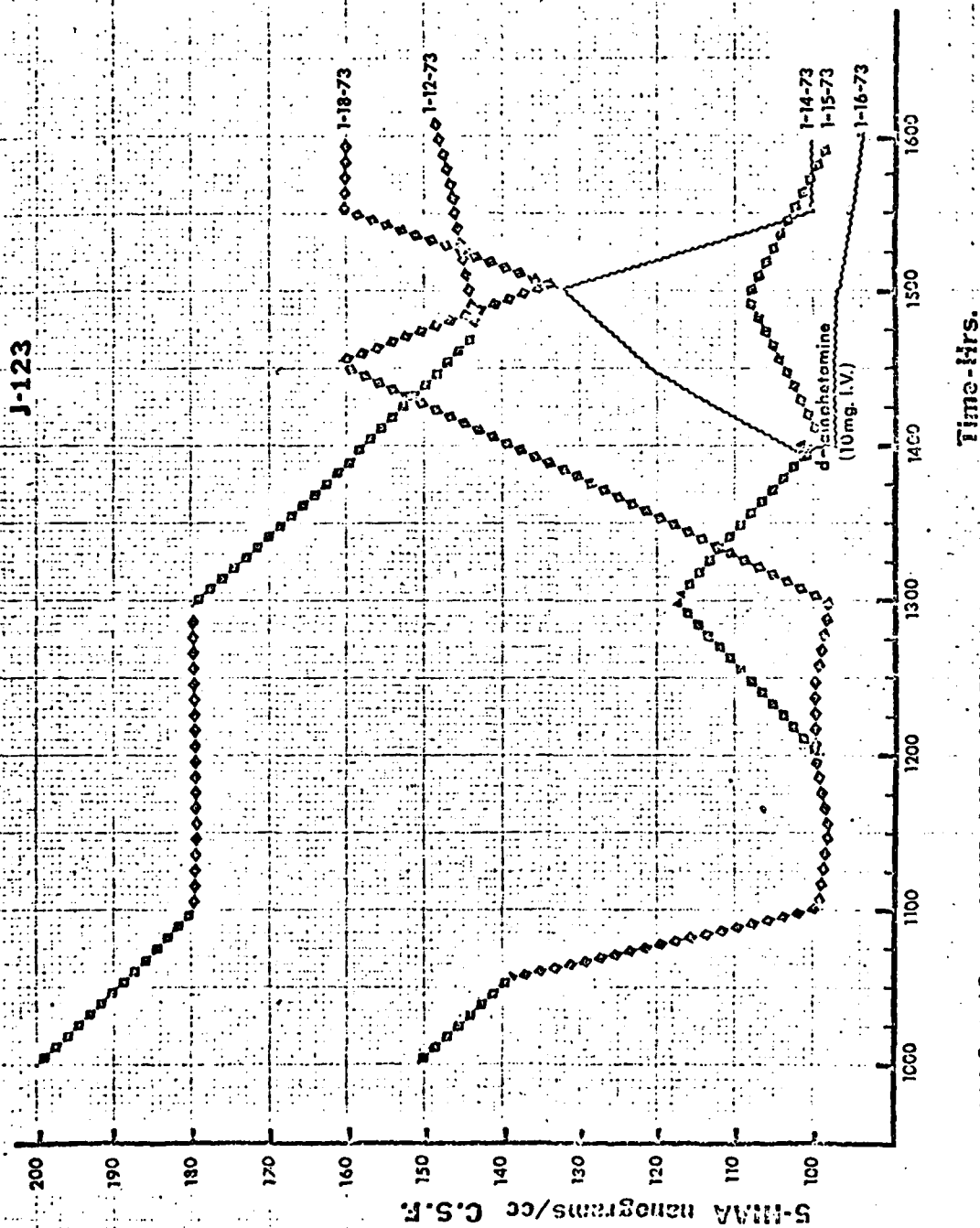


FIGURE 11. A comparison of drug days and control days in the 5-HIAA response to d-amphetamine.

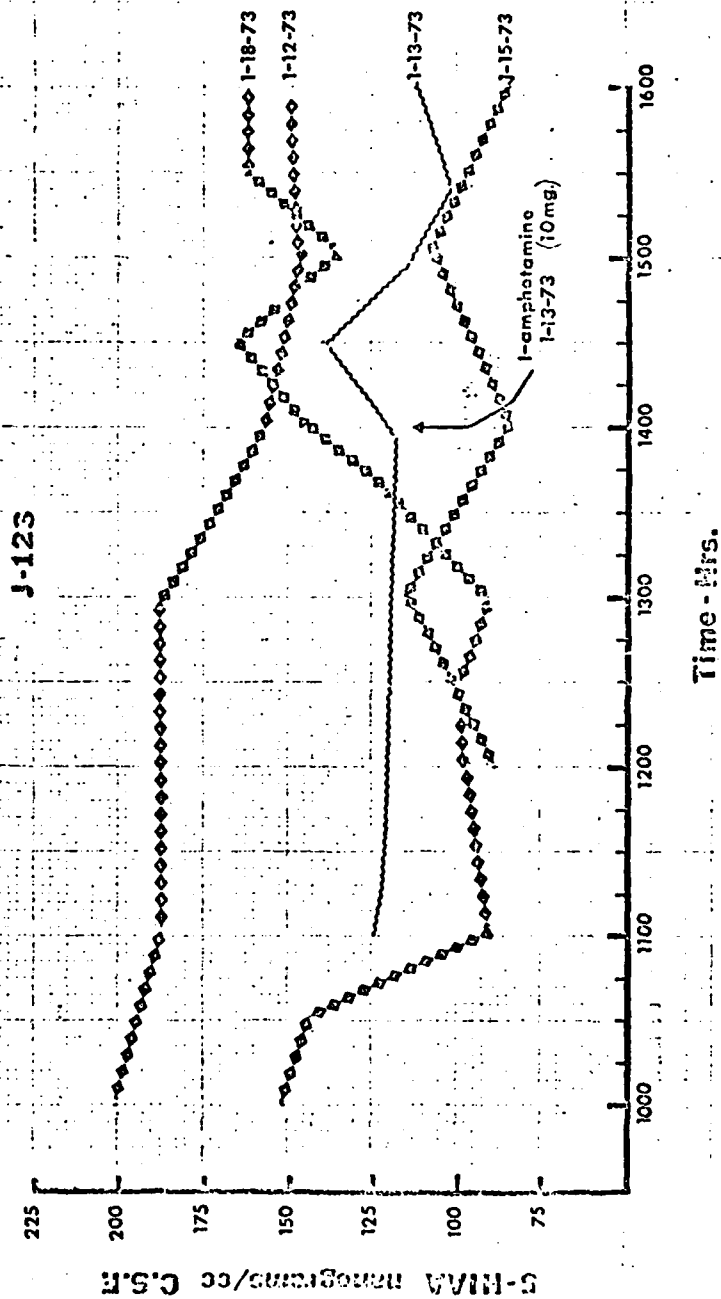


FIGURE 12. A comparison of drug and control days in the 5-HIAA response to 1-ampphetamine.

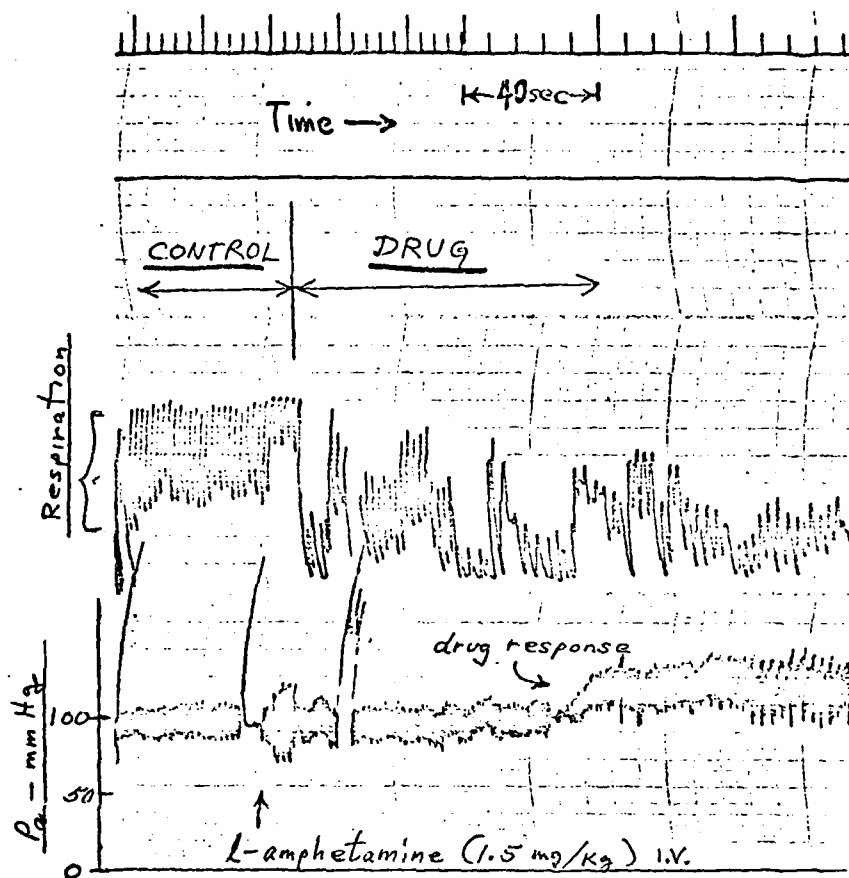


FIGURE 13. Recording of the circulatory and respiratory response to l-amphetamine (1.5 mg/kg I.V.) from experiment in Fig. 8. Blood pressure (P_a).

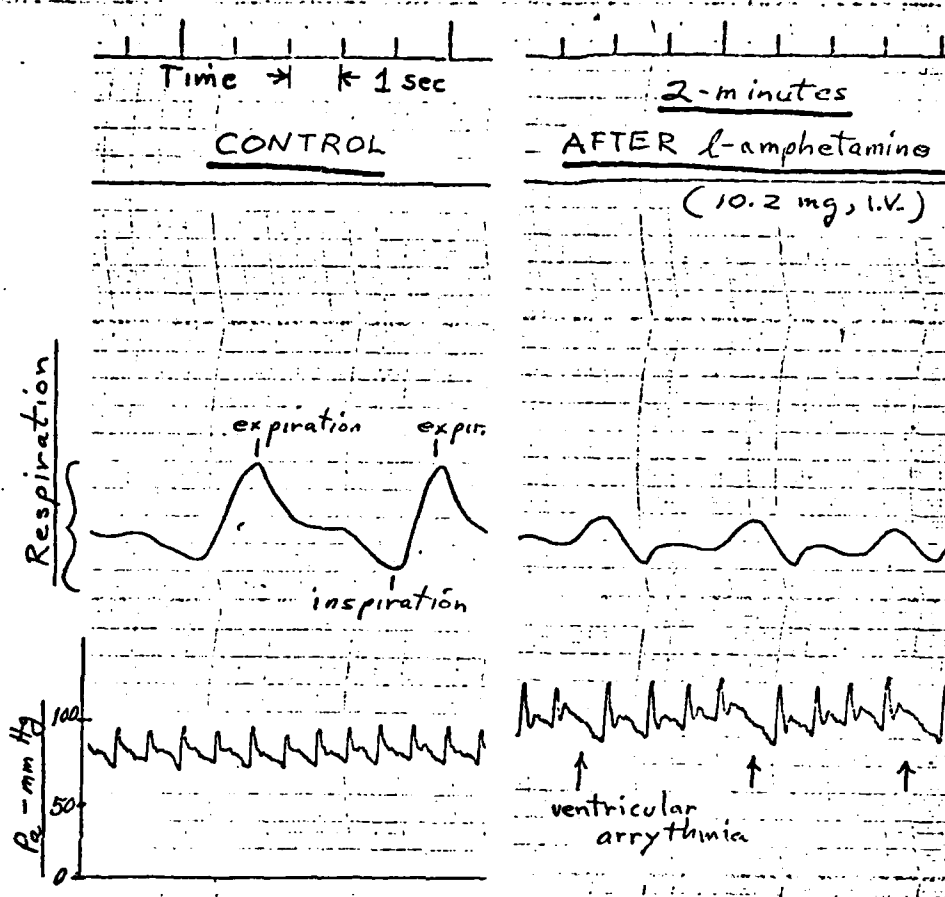


FIGURE 14. Recording of circulatory and respiratory adjustments to l-amphetamine from Fig. 13, recorded at faster chart speed. Note the increased respiratory rate and decreased tidal volume associated with either d- or l-amphetamine at 1.5 mg/kg I.V. Some cardiac arrhythmia was apparent following the l-isomer, but was never seen following the d-isomer - again indicating some stereoselectivity in direct myocardial effects. We do not know if the arrhythmia was due to PVC's or not, but the inclusion of a simultaneous ECG in work proposed under the contract renewal should be of great value in better delineating cardiac function.

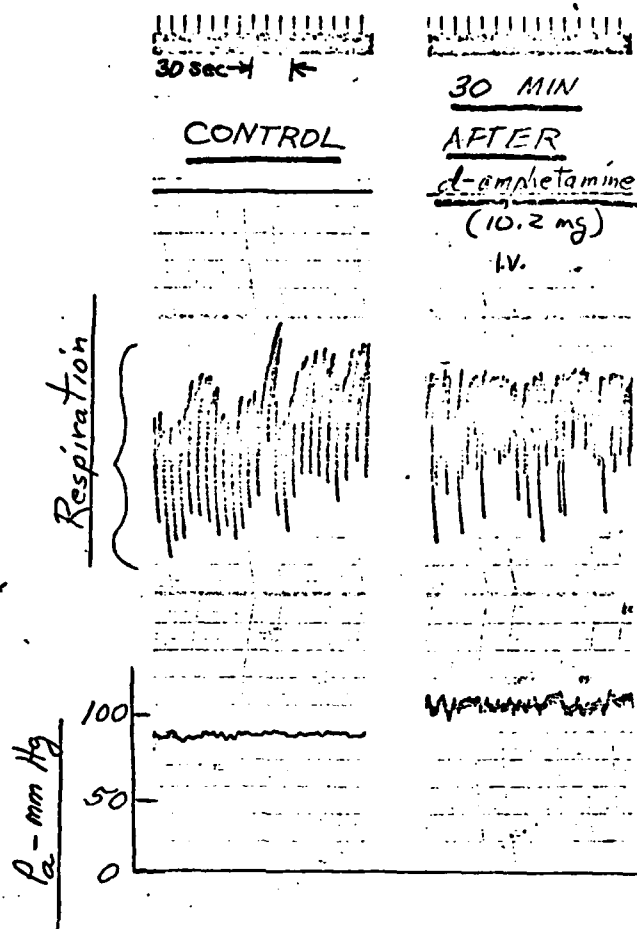


FIGURE 15. Circulatory and respiratory effects of d-amphetamine 1.5 mg/kg, I.V. Only mean blood pressure is shown. Note the increased respiratory frequency here as in Fig. 14 after the 1-isomer. Arterial pressure (P_a). Data is recorded from experiment in Fig. 7.

Levels of HVA in the CSF are decreased by the administration of d-amphetamine, whereas levels of HVA remain essentially unchanged after a similar dose of the l-isomer on separate days. Levels of 5-HIAA are also slightly decreased following the d-amphetamine, with a lesser effect after the l-isomer (Figs. 7,8).

Figs. 9 and 10 show the normal variations in CSF levels of HVA on separate control days, compared with the response obtained on drug days. Figs. 10 and 11 indicate the variations observed in CSF levels of 5-HIAA on separate control days, and show a considerable scatter. Possible reasons for this scatter are presented in the discussion. It is sufficient to say here that we feel the scatter may be a reflection of the CSF sampling procedure, and we are attempting to refine the method of taking the samples by incorporating a remote, automated sampling device.

We have conducted one amphetamine toxicity study, in which the animal three successive 10 mg I.V. doses of d-amphetamine spaced at hourly intervals (Fig. 16); and these data are qualitatively similar to the single injection experiments. We stopped the injections after 30 mg total because of large fluctuations in diastolic blood pressure and severely distorted respiratory patterns (not shown) observed on the monitor oscilloscope.

Inhibition of a tissue weak acid transport system by Probenecid (200 mg/kg I.P.). In a series of preliminary experiments, we have explored the possibility of using Probenecid to estimate the turnover rates for serotonin. The rate constant for the appearance of 5-HIAA in the CSF after inhibition with Probenecid is proportional to the rate of formation of serotonin (and release). It is apparent from Figs. 17 and 18 that the appearance rates for 5-HIAA in separate experiments are fairly similar, and would allow direct comparisons of drug effects on turnover by giving the drug at 1 hr after the initial Probenecid injection. In future studies we will be able to give the Probenecid I.V. thus attaining more nearly "instant" inhibition of the transport system. In these studies both HVA and 5-HIAA seem to be affected by Probenecid. We would like to explore the possibility of measuring dopamine turnover by this method as well.

DISCUSSION

The most striking finding of the drug effects was that amphetamines clearly affected three separate neural systems, two of which responded in a stereoselective manner (HVA and 5-HIAA). Both the d- and l-isomers manifest peripheral cardiovascular effects however only the d-isomer is able to promote behavioral stereotypy which is characteristic of amphetamine psychoses and varies in nature with the species concerned. This is the same sort of purposeless, searching behavior shown by human amphetamine addicts. We tentatively presume that several factors are operative in the amphetamine response, on the basis of the data presented. MAO inhibition after d-amphetamine is evidenced by the decreases in both HVA and 5-HIAA. The presence of behavioral stereotypy suggests an increased release of dopamine after the d-isomer, perhaps accompanied by a decreased

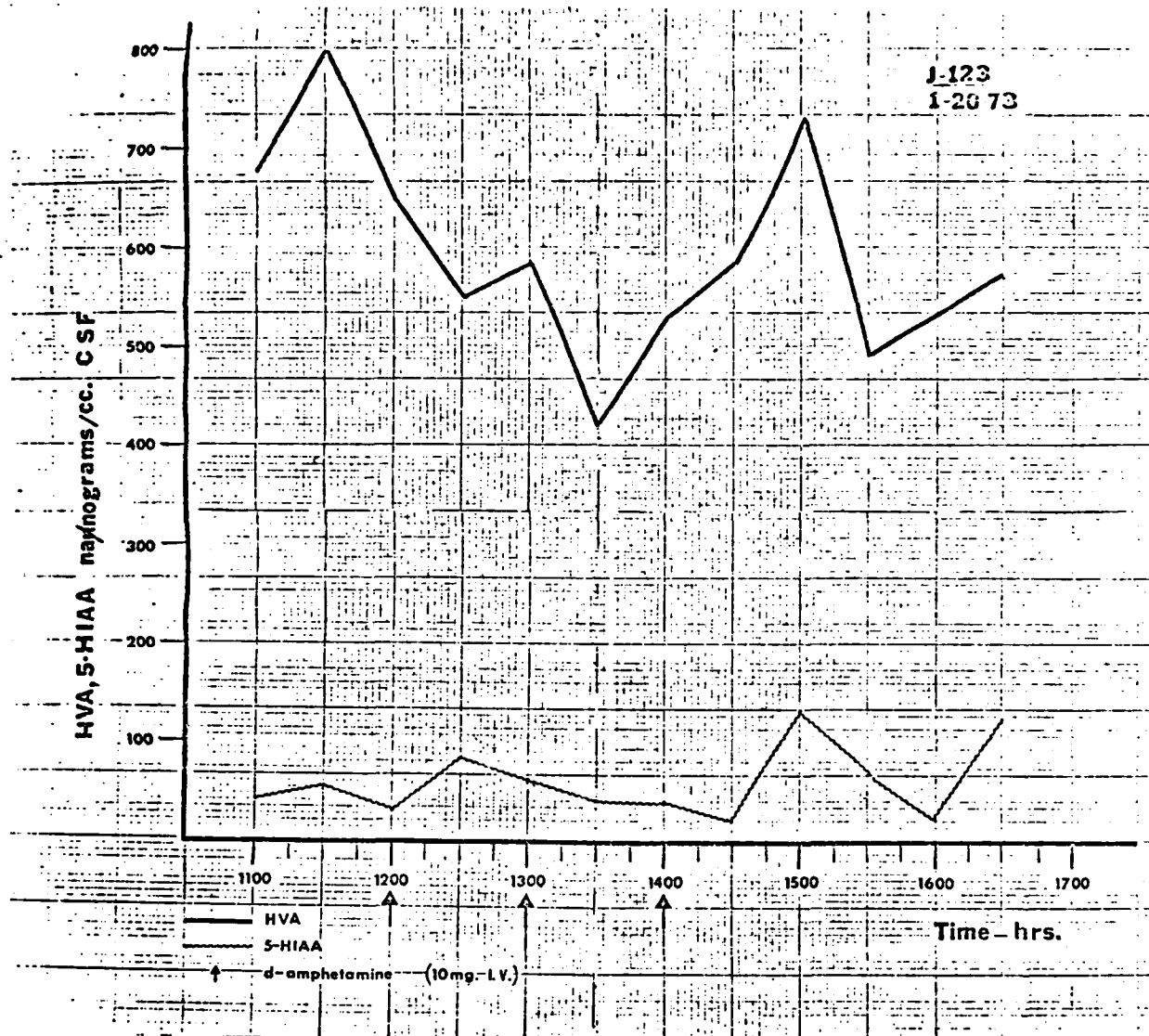


FIGURE 16. Results of a d-amphetamine "toxicity study." Three separate injections of 10mg amphetamine were given starting at 1200 and spaced at 1 hour intervals. There is a trend to decreased levels of HVA consistent with the single dose studies shown in Fig. 10. This represents sub-arachnoid CSF, and this experiment should be repeated while sampling ventricular CSF.

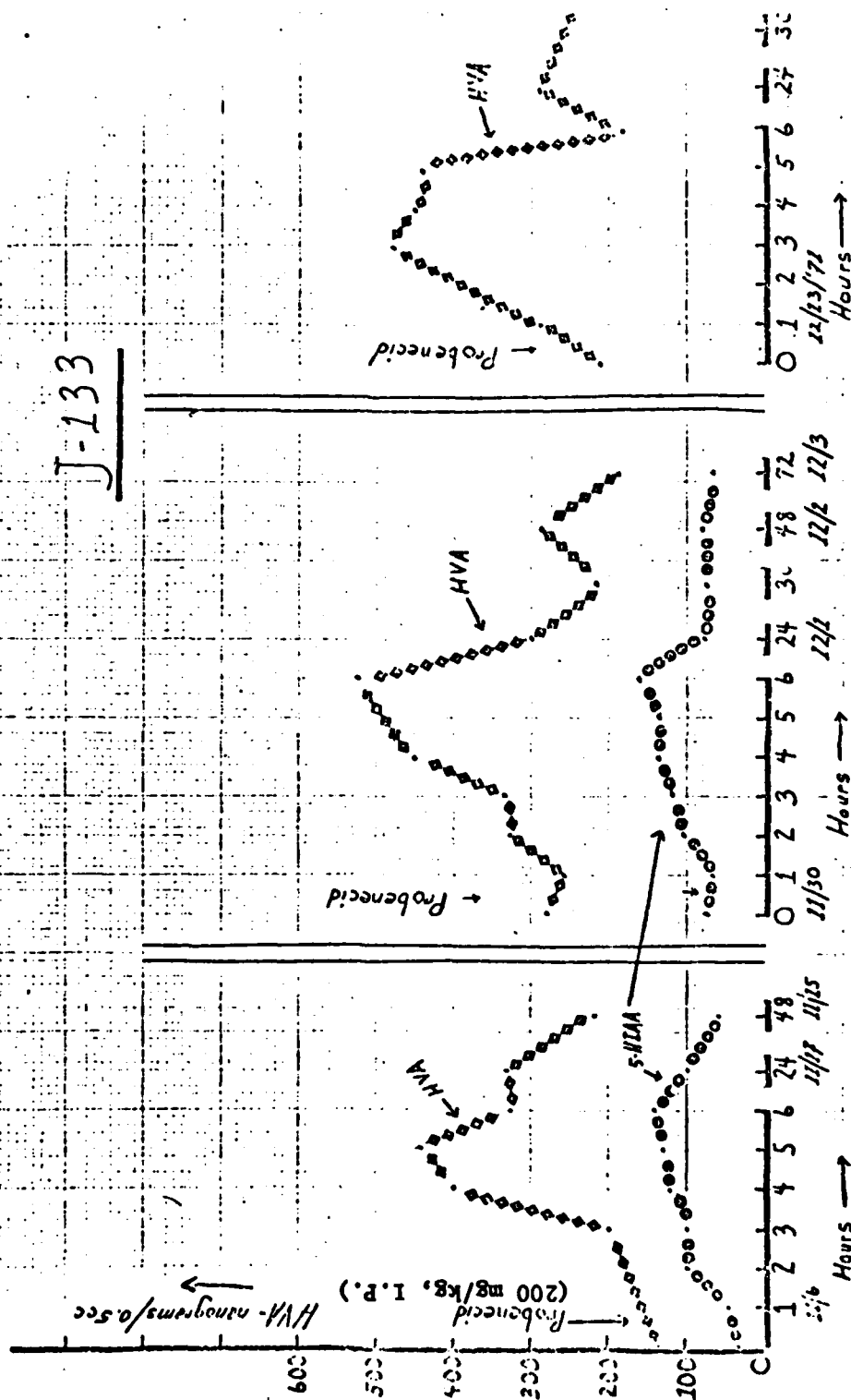


FIGURE 17. Effects of Na Probenecid on cisternal levels of HVA and 5-HIAA using the Ommaya reservoir (Fig. 1E)

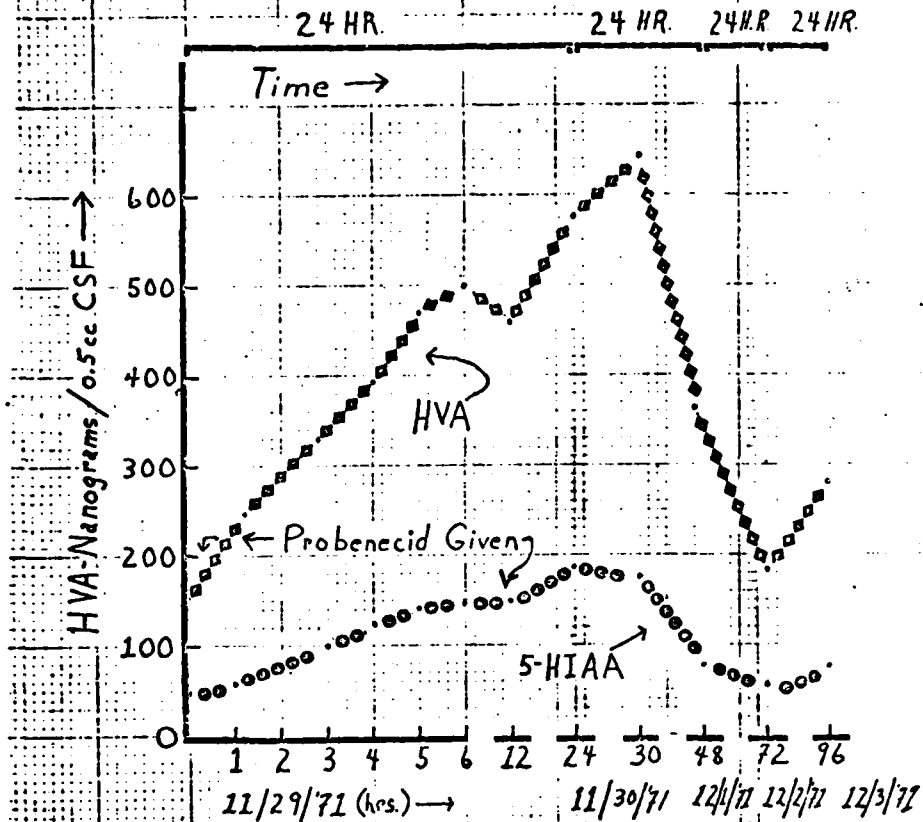


Figure 18. One additional study on the effects of Na Probenecid on cisternal CSF levels of HVA and 5-HIAA using the Ommaya reservoir preparation. Initial dose was 200 mg/kg I.P., followed by a second injection at 12 hours after the first.

re-uptake of dopamine by the presynaptic endings simultaneously. By simple mass action effects, there should be increased o-methylation of the dopamine present in the synaptic cleft and this should be reflected by increases in the CSF of the o-methylated metabolite (3-methoxy dopamine). We did not measure the CSF levels of 3-methoxy dopamine, but we would predict a significant elevation following the d-amphetamine and relatively little change after l-amphetamine on the basis of our data.

Since we did not measure actual levels of central norepinephrine metabolites, our only indication of activity in these neurons is given by the observed changes in blood pressure, heart rate, respiration and general restlessness. Our data suggest that the norepinephrine neurons may be more or less equally affected by both isomers, but this is speculation.

The serotonin system seems to be more affected by the d-isomer than the l-isomer, although conclusions are hard to draw because of the large overall variations in levels of 5-HIAA and the interfering presence of the transport system specific for 5-HIAA in the region of the fourth ventricle or brain tissue proper.

While it is experimentally difficult to distinguish between the two processes of release and reuptake in terms of drug effects, the use of such drugs as cocaine which block the catecholamine reuptake process may prove useful once more definitive data on the drug effects themselves has been obtained - as proposed in the contract renewal application. An additional test of the drug effects (amphetamines) on different neuronal populations would be to give drugs which block the conversion of dopamine to norepinephrine, and observe whether the cardiovascular effects were still present in association with the stereotyped behavior. A reduction in the magnitude of the cardiovascular effects by such pretreatment followed by d- amphetamine would indicate that norepinephrine neurons were partly responsible for the cardiovascular effects and that dopamine neurons were primarily responsible for the behavioral stereotypy.

Once we have a preparation in which we can clearly distinguish drug effects on different neuronal populations, then it will be possible to test a wide variety of drugs and antagonists for the basic biologic mechanisms of action, and in addition to screen new drugs for their abuse or therapeutic potential. The ability to measure selective actions of drugs on different catecholamine systems has obvious practical importance in developing and testing possible antagonists with selective actions on specific catecholamine systems. By the use of stereoisomers of drugs (including opiates) it may be possible to distinguish between specific and non-specific binding if it can be shown that the in-vivo pharmacologic activity parallels the in-vitro relative binding for the stereoisomers. These studies would involve the use of radioisotopes and may be the topic of a future contract proposal. For the present effort we feel that a study of possible effects of opiates and other drugs on serotonin turnover may be a profitable and worthwhile attempt to determine the possible drug effects on serotonin neurons.

CONSLUSIONS

We conclude that treatment of chronic devices implanted in the ventricles or sub-arachnoid space with a TDMAC-Heparin complex will significantly reduce the rate of tissue growth on the surface of such devices through increased tissue compatability.

On the basis of the work in this annual report, we feel that the monkey model we have developed, which permits several types of simultaneous physiologic measurements, will be especially valuable in evaluating basic mechanisms of action of commonly abused drugs, antagonists and perhaps to screen new compounds for abuse potential or use as therapeutic agents capable of blocking the abused drug effects.

Concerning the drugs studied to date, we have been able to distinguish separate effects of d- and l-amphetamine isomers on distinct neural transmitter systems. These studies are only preliminary, and will require the addition of more specific physiologic measurements to evaluate the total in-vivo drug action.

RECOMMENDATIONS

We recommend that the studies of CSF biochemical response to administration of the abused drugs be continued, and be expanded to incorporate measurements of circulatory and respiratory variables in the chronic monkey preparation. Since the secondary effects of respiratory and circulatory disturbances may exert profound effects on neurotransmitter metabolism and release via a number of mechanisms, we feel that it is essential to determine the exact nature of the contribution of these variables to the in-vivo drug response, both biochemically and behaviorally. The respiratory and circulatory systems can act as sensitive built-in indicators of drug action and response and can provide information on drug effects not otherwise obtainable through usual chemical analyses.

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APPENDIX - A

Foreword:

The material in this appendix is intended to supplement the main body of the report with information obtained during the last quarter from experiments which are continuing at the present time (Year II). During the last quarter we conducted additional tests of d-amphetamine, and began 24 hour collection periods of CSF to investigate possible diurnal variations in the CSF metabolites of interest. In addition, as a prelude to the following year, we began circulatory studies of the opiates methadone and metyrapone. These circulatory studies were conducted acutely on dogs because they are easier to obtain and considerably less expensive than primates, and the experiments were of a terminal nature. We do not have the results of the analyses on the monkey CSF yet, so this short section will be devoted exclusively to the acute dog studies.

Materials and Methods:

Healthy, adult mongrel dogs (20 kg) were anesthetized with a barbituate (Surital 40 mg/kg initial and 15 mg/kg/hr sustaining), intubated, artificially ventilated, and catheters placed in the following locations: left ventricle, right atrium, pulmonary artery and femoral artery. End-inspiratory pressure was monitored as an index of total thoracic compliance, and a standard lead-II electrocardiogram was taken. Lateral and dorsal chest X-rays were taken during the control and experimental periods. At the end of the experiment, the lung was removed, divided into the distinct lobes, weighed and then freeze-dried 24 hr and weighed again to determine extravascular lung water. Methadone (2 mg/kg, I.V.) was given to these dogs at time T=0, and they were sacrificed at T=60 min.

In a separate experiment, utilizing an isolated, perfused beating dog heart, an oxygen micro-electrode was inserted into the left ventricular myocardium to record tissue PO_2 at a constant blood flow rate and outflow pressure. This experiment was designed to test the possible mechanism of action of opiates on the myocardium. The previous studies with methadone had suggested a pronounced direct effect on left-ventricular contractile force, and we reasoned that this effect may be mediated by variations in the extracellular tissue PO_2 , related to administration of any one of a family of drugs (opiates).

Results: (n = 5)

1. Methadone: In the artificially ventilated dog, intravenous methadone at 2 mg/kg exerts a pronounced, long-lasting effect on left ventricular contractile force (nearly 50% reduction), and subsequently cardiac output. One typical experiment of this type is shown in Fig. 19A. Pulmonary compliance does not change over the next hour, however there is a tendency to increase slightly which is not significant. Femoral arterial pressure is decreased, and right atrial and pulmonary arterial pressure varied only slightly in most instances. The (A-V) blood PO_2 gradients and content differences increased 10-20%, most likely as a reflection of the reduced cardiac output associated with the striking decrease in left ventricular systolic pulse height.

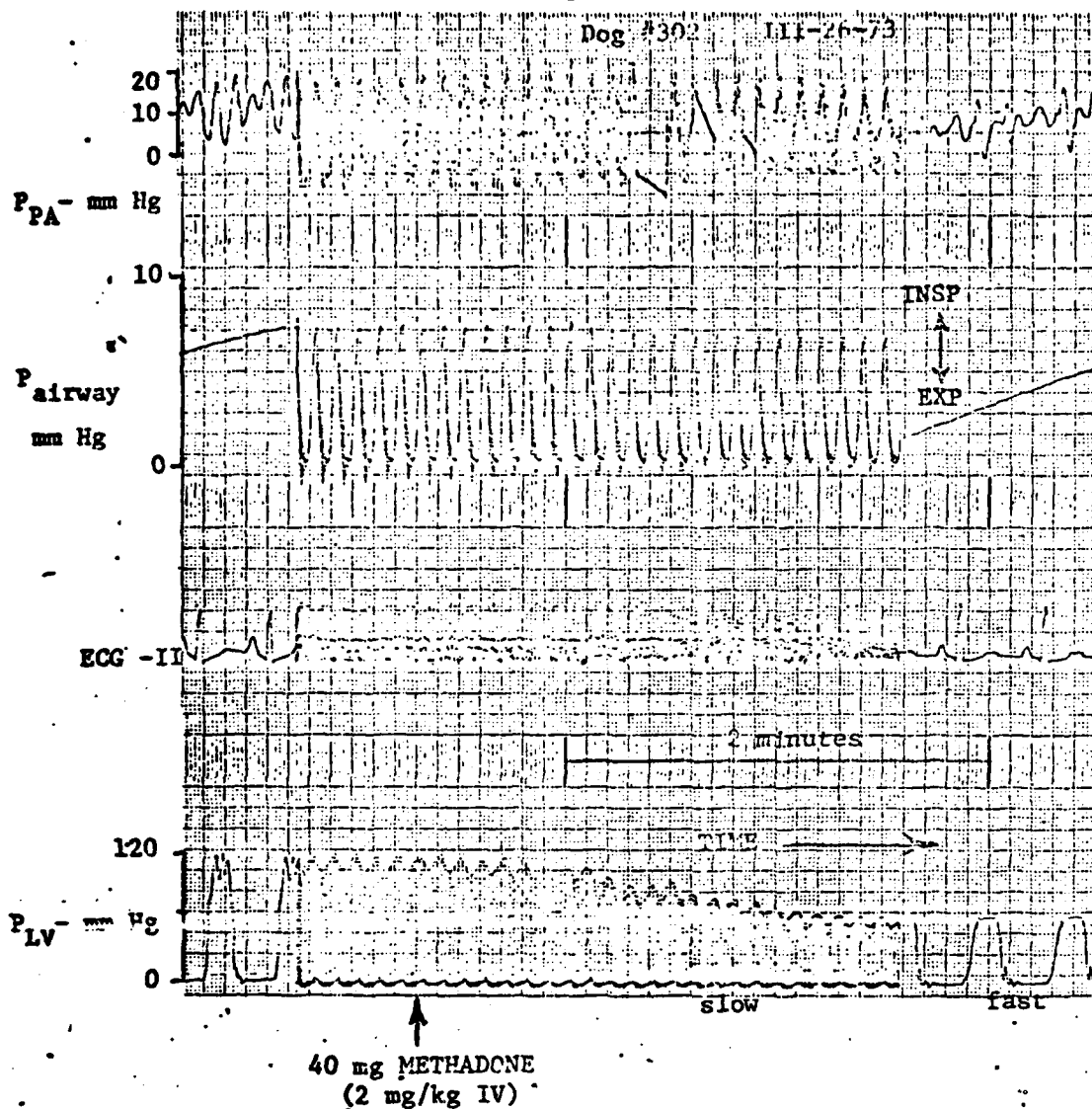


Figure 19A. Record from a typical experiment using methadone. Note the striking decrease in left ventricular force of contraction (P_{LV}), as indicated by the 50% reduction in systolic pulse height. Primary effect is on the myocardium, and so long as the animal is artificially ventilated there is no change in lung compliance (P_{airway}) which might result from pulmonary edema. Lung water was normal in this dog at one hour (75-78%), and there was practically no recovery of left ventricular function for the duration of the experiment. Arterial pressure (not shown) was decreased in accordance with changes in P_{LV} .

In order to examine the basic mechanism of the effect of methadone on the left ventricular myocardium in greater detail, a separate experiment was conducted using an isolated heart preparation. This preparation is maintained at a constant flow, and there is no possibility of CNS interaction with left ventricular function. A polarographic oxygen electrode (Platinum) was placed in the left ventricular myocardium between the anterior descendens and circumflex arteries, at a depth of 2 mm. The drug used in this study was metyrapone, similar chemically to methadone. The results are in the following figure (19B).

ISOLATED DOG HEART PREPARATION

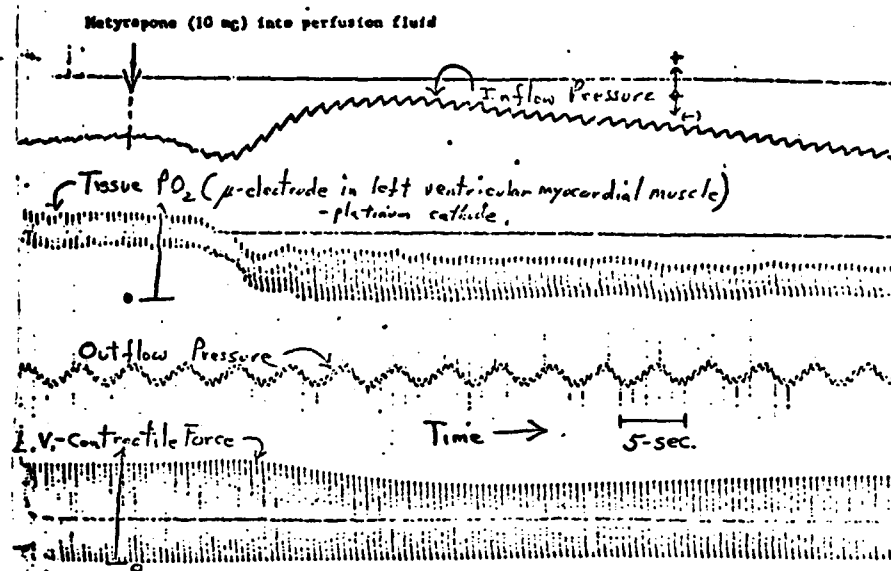


Figure 19B. Record from isolated heart preparation. Note the decrease in left ventricular (LV) contractile force, similar to the experiment with methadone on the intact dog. Also note that the decline in contractile force is preceded by a fall in the interstitial PO_2 (extracellular). Outflow venous pressure remains constant, and inflow pressure recovers while tissue PO_2 remains low.

It would appear on the basis of these experiments that one action of opiates and related compounds on the peripheral circulation is manifested through a decrement in left-ventricular function; and further, the effect on the left-ventricular myocardium is somehow related to a reduced oxygen availability and subsequent shifting of the left-ventricular function curve to the right, reducing cardiac output and arterial pressure. These effects would act in combination with any central CNS effects or reflexes if in fact the cerebral circulation is compromised. Additional studies are being conducted to test the effects of these and other drugs on cerebral blood flow while CSF is sampled simultaneously.

There are two possible explanations for these results: First, the drugs may have a vaso-active effect, redistributing the intramyocardial circulation; or secondly, the drugs may effect a reduction myocardial cellular function through a direct action on the active heart cells by interfering with mitochondrial energy production (Krebs cycle). It is not possible at this time to distinguish between these two possibilities. All we can say is that the tissue PO_2 , whether it is intracellular or extracellular, decreases after the drug. Perhaps there is an oxygen transport system within the myocardium which the drugs are inhibiting.

If such an oxygen transport system exists within the myocardium, it may also be present within the brain, and its presence would have far reaching implications indeed in the interpretation of drug effects on neural function.

Unclassified

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Security Classification			DOCUMENT CONTROL DATA - R & D		
(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)					
1. ORIGINATING ACTIVITY (Corporate author)			24. REPORT SECURITY CLASSIFICATION		
Department of Environmental Medicine The Johns Hopkins University Baltimore, Maryland 21205			Unclassified		
2. REPORT TITLE			25. GROUP		
6 Biochemical Analysis of Cerebrospinal Fluid Changes in Response to Drug Administration.					
4. DESCRIPTIVE NOTES (Type of report and inclusive dates)					
Final Report, May 1, 1972 - May 1, 1973					
5. AUTHOR (Last name, first name, last name)					
10 Barry Burns Ph.D., Principal author Joseph V. Brady Ph.D.					
9. REPORT DATE		76. TOTAL NO. OF PAGES		75. NO. OF REFS	
Sept. 11, 1973		40		3	
89. CONTRACT OR GRANT NO.		98. ORIGINATOR'S REPORT NUMBER(S)			
Contract No. DADA17-72-C-2119		Report No. 02			
8. PROJECT NO.		99. OTHER REPORT NO(S) (Any other numbers that may be assigned to this report)			
		11 11 Sep 73 12 142			
10. DISTRIBUTION STATEMENT					
Distribution Unlimited					
11. SUPPLEMENTARY NOTES			12. SPONSORING MILITARY ACTIVITY		
			U.S. Army Medical Research and Development Command Washington, D.C. 20314		
13. ABSTRACT					
<p>We have successfully designed and tested a chronic, remote sampling system for CSF with a total dead space of less than 0.06 ml. A monkey model has been developed for the study of drug abuse while monitoring behavior, CSF neurochemical changes and cardiovascular effects of selected drugs. The first series of drug studies has been completed using the d- and l-stereoisomers of amphetamine. Both isomers at levels of 1.5 mg/kg effect the norepinephrine neuronal system as evidenced by the pronounced peripheral respiratory and cardiovascular effects. However at this same dosage level the d- isomer results in striking behavioral changes associated with dopaminergic neurons. The l- isomer does not produce any stereotyped behavior and only creates general conditions of restlessness. We found that levels of the serotonin metabolite 5-HIAA in the CSF were essentially unchanged by either amphetamine isomer although there was a greater tendency to decrease after the d- isomer than the l- isomer. CSF levels of a dopamine metabolite HVA decreased significantly after the d- isomer indicating possible increased rates of release of dopamine, reduced reuptake of dopamine and increased possible o-methylation to 3-methoxy dopamine. These changes in HVA with the d-isomer are supported by the simultaneous tendency for CSF levels of 5-HIAA to decrease and support the hypothesis of MAO inhibition. Levels of HVA in the CSF were essentially unaffected by the l-amphetamine isomer at the same dosage levels. (U)</p> <p>We observed significant changes in peripheral cardiovascular and respiratory systems which would be associated with norepinephrine neurons and which were produced by both amphetamine isomers with nearly equal effectiveness. Behavioral changes were of a type which indicate that dopamine neurons were selectively affected by the d-isomer and not the l-isomer. We conclude our model is valuable for drug studies. (U)</p>					

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KEY WORDS	LINK A		LINK B		LINK C	
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Drug Abuse						
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